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SYSTEMIC AND CARCINOGENIC TOXICITY INFORMATION FOR BENZENE, CARBON TETRACHLORIDE, CHLORETHANE, 1,2-DICHLOROETHANE, KEPONE, PERCHLOROETHYLENE, AND STYRENE - (USED AS A REFERENCE IN OU5 RI REPORT - APPENDIX A)

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
CINCINNATI, OHIO 45268

MEMORANDUM

DATE: September 21, 1994

SUBJECT: Systemic and Carcinogenic Toxicity Information for Benzene (CASRN 91-20-3) Carbon Tetrachloride (CASRN 56-23-5), Chloroethane (CASRN 75-00-3), 1,2-Dichloroethane (CASRN 95-50-1), Kepone (CASRN 143-50-0), Perchloroethylene (CASRN 127-18-4) and Styrene (CASRN 100-42-5) (Fernald Environmental Management Project, Operable Unit #5/Fernald, Ohio)

FROM: Joan S. Dollarhide
Director
Superfund Health Risk Technical Support Center
Chemical Mixtures Assessment Branch

TO: Pat Van Leeuwen
U.S. EPA
Region V

This memorandum is in response to a request submitted by Elaine Merrill of FERMCO, for oral and inhalation systemic and carcinogenic toxicity information for benzene, carbon tetrachloride, chloroethane, 1,2-dichloroethane, kepone, perchloroethylene and styrene for the Fernald Superfund site.

Attached please find the following information:

Attachment 1: *Risk Assessment Issue Paper for: Derivation of a Provisional Chronic Inhalation RfC for Benzene (CASRN 71-43-2)*

Attachment 2: *Risk Assessment Issue Paper for: Derivation of a Provisional Inhalation RfC for Carbon Tetrachloride (CASRN 56-23-5)*

Attachment 3: *Risk Assessment Issue Paper for: Toxicity and Carcinogenicity of Chloroethane (Ethyl Chloride) (CASRN 75-00-3)*

Attachment 4: *Risk Assessment Issue Paper for: Derivation of a Provisional Inhalation RfC for 1,2-Dichloroethane (CASRN 107-06-2)*

Attachment 5: *Risk Assessment Issue Paper for: Carcinogenicity Information for Tetrachloroethylene (perchloroethylene, PERC) (CASRN 127-18-4)*

Attachment 6: *Risk Assessment Issue Paper for: Carcinogenicity Information for Styrene (CASRN 100-42-5)*

Please feel free to contact the Superfund Health Risk Technical Support Center at (513) 569-7300 if you have any questions or need additional information.

Attachments

cc: E. Moran (Region V RTIC)
E. Merrill (FERMCO)

Attachment 1

(3/23/94)

Risk Assessment Issue Paper for:
Derivation of a Provisional Chronic Inhalation RfC
for Benzene (CASRN 71-43-2)

INTRODUCTION

To identify research reports pertinent to the derivation of a provisional chronic RfC for benzene, EPA and ATSDR documents on benzene (U.S. EPA, 1980; 1984; 1989; 1993; 1994a,b,c; ATSDR, 1991) and the HSDB, RTECS, and TSCATS databases were reviewed; in addition, a computer search of the literature was conducted (TOXLINE, June, 1986 - February, 1992).

OSHA lists an PEL TWA of 1 ppm; however, some segments of industry are exempt from the 1 ppm standard, and instead have a PEL TWA of 10 ppm (OSHA, 1989). ACGIH lists a TLV TWA of 10 ppm; however a TLV TWA of 0.1 ppm has been proposed and is awaiting verification (ACGIH, 1991). The NIOSH REL (10-hour TWA) is 0.1 ppm (NIOSH, 1991).

TOXICITY IN HUMANS

There is an extensive database on the toxicity of inhaled benzene. Secondary sources, as well as literature searches, were used to identify studies that defined the thresholds of toxicity. Specifically, we looked for studies that evaluated subchronic, chronic, developmental, and reproductive toxicity of inhaled benzene. When numerous studies were available, we chose those for which toxic effects were observed at low concentrations of benzene. A number of epidemiology studies were available regarding chronic toxicity of inhaled benzene; four are reported herein, covering a wide range of exposure levels and effects.

Aksoy et al. (1971) examined hematological parameters in 217 apparently healthy male workers (mean age 24.7 years) exposed to 30-218 ppm benzene (96-696 mg/m^3) for 3 months to 17 years, and in 100 male hospital workers and medical students (mean age 26.6 years). Peripheral blood samples were obtained for measurement of RBC, WBC, PCV, platelets, and differential counts. In 11 benzene-exposed workers known to have hematological abnormalities, bone marrow samples were obtained for determination of cellularity and myeloid and erythroid series. Twenty-four percent of the exposed workers had hematological abnormalities, including leukopenia (9.7%), thrombocytopenia (1.84%), leukopenia associated with thrombocytopenia (4.6%), pancytopenia (2.76%), acquired pseudo-Pelger-Huet anomaly (0.46%), lymphocytosis (0.46%), giant platelets (0.46%), eosinophilia (2.3%), basophilia (0.46%), and eosinophilia associated with basophilia (0.46%). Low hemoglobin levels, PCV,

and MCV, indicative of mild or moderate hypochromic or normochromic anemia, were observed in 33% of the benzene-exposed workers. In bone marrow tests, 9/11 workers had hematopoietic abnormalities, including hypercellularity (in 1 worker), hypocellularity (4), and maturation arrest (8) and vacuolization (4) in the erythroid and myeloid series. This study identifies a LOAEL of 30 ppm (96 mg/m^3) for hematopoietic effects in humans.

Kipen et al. (1989) examined about 18,000 peripheral blood counts from hematologic surveillance records on 459 workers employed in the rubber industry between 1940 and 1975. Mean concentrations of benzene decreased from 137 to 66 ppm between 1940 and 1948, with a mean 8-hour TWA of 75 ppm (239.6 mg/m^3). WBC counts increased from 1940-1948 and were positively correlated with the decreasing benzene levels. Between 1948-1975 workers were exposed to mean 8-hour TWA concentrations of 15-20 ppm (48-64 mg/m^3). WBC counts in blood samples from workers exposed from 1948-1975 were not correlated with changing benzene exposure levels. These data suggest that benzene exposure in the 75 ppm (239.6 mg/m^3) range influences WBC count in exposed workers, whereas exposure to benzene at 15-20 ppm (48-64 mg/m^3) does not influence WBC count.

Collins et al. (1991) examined hematological parameters (peripheral blood RBC, WBC, hemoglobin, platelets, and MCV) in workers (n=200) exposed to benzene over a 10-year period. Within this 10-year period the mean length of exposure was 7.3 years. The workers were exposed to an 8-hour TWA of 0.01-1.40 ppm benzene. The mean TWA exposure was 0.045 ppm (J. Collins, 1992, personal communication). A group (n=268) of non-benzene exposed workers in the same plant were used as controls. There were statistically significant differences on demographic (age, race, sex) and personal habit (currently smokers, regular exercise) variables between the benzene-exposed workers and the control group. Multiple regression analyses were applied using the confounding factors and current exposure as independent variables. No significant correlations between cumulative exposure and hematological parameters were identified. Thus, this study identifies a free-standing NOEL of 0.045 ppm (0.14 mg/m^3) for hematological effects in humans.

Fishbeck et al. (1978) examined hematological parameters (RBC, WBC, hematocrit, hemoglobin, mean corpuscular volume, platelets, differential blood counts, clot retention determinations, sedimentation rate, and blood indices) in 10 employees exposed to high benzene concentrations [8-hour TWA of >25 ppm (>80 mg/m^3)] for 2.5-22.9 years, with an average of 9.6 years of exposure. Concentrations of benzene in the work area were especially high in 1963, with the 8-hour TWA ranging from 37-132 ppm (118-422 mg/m^3); after 1963, conditions were altered to assure that concentrations of benzene remained below 25 ppm (80 mg/m^3) (the acceptable limit at that time). Examination of the 10 employees in 1963 revealed enlarged RBC's, high MCV (10/10), slightly low hemoglobin levels (9/10), and transient anemia; bone marrow was examined at this time and no abnormalities were found. After 1963, hematological values for these employees improved (in 1977, 5/10 workers had increased MCV values) and by 1978 none of the employees had developed serious health problems. The authors concluded that exposure of workers to high levels of benzene produced transient hematological effects, which did not influence the long-term overall health of the workers.

TOXICITY IN ANIMALS

Male C57BL/J mice (sample size not reported; initial age 8 weeks) were exposed via inhalation to vapor concentrations of 0 or 10 ppm benzene (0 or 32 mg/m^3) for 6 hours/day, 5 days/week for up to 178 days (Baarson et al., 1984). After 32, 66, and 178 days of exposure, peripheral blood samples were obtained from all mice for determination of levels of RBC, lymphocytes, and neutrophils, and 5 mice/exposure level were sacrificed for measurement of erythroid progenitor cells [colony forming units - erythroid (CFU-E), burst forming units (BFU-E), nucleated red cells, and total cellularity] in bone marrow and spleen. There was a significant ($p<0.05$) decrease in levels of RBC (at 66 and 178 days) and lymphocytes (at all sampling times) in peripheral blood of benzene-exposed mice. CFU-E and BFU-E in bone marrow were significantly ($p<0.01$) decreased at all sacrifice times and at 66 days, respectively; after 178 days of treatment, bone marrow CFU-E was 5% of controls. Splenic CFU-E (10% of controls), nucleated red cells (15%), and total nucleated cellularity (84%) were significantly ($p<0.05$) decreased in mice sacrificed at 178 days. This study identifies a LOAEL of 10 ppm (32 mg/m^3) for depressed hematopoiesis in mice.

Male CD-1 mice (11-12/exposure level/exposure duration; 8-12 weeks of age) were exposed for 6 hours/day, 5 days/week to vapor concentrations of 0 or 9.6 ppm (0 or 31 mg/m^3, respectively) benzene for 10 weeks or to 0 or 302 ppm (0 or 966 mg/m^3) benzene for 26 weeks (Green et al., 1981a,b). On the day of the last exposure, samples (pooled from groups of 3-4 mice) were obtained from the peripheral blood, bone marrow and the spleen to evaluate hematological and hematopoietic cells. In mice exposed to 9.6 ppm (31 mg/m^3), no adverse effects were observed with respect to mortality, body weights, or cells in the peripheral blood or bone marrow. Splenic weight, total nucleated cells per spleen, and nucleated red blood cells per spleen were significantly ($p<0.05$) increased in mice exposed to 9.6 ppm (31 mg/m^3). Mice exposed to 302 ppm (966 mg/m^3) had the following significant ($p<0.05$) changes: increased mortality rate; decreased numbers of lymphocytes and RBC in peripheral blood; decreased numbers of lymphocytes, granulocytes, multipotential hematopoietic stem cells, and committed granulocyte/macrophage progenitor cells in bone marrow; decreased splenic weight, and numbers of lymphocytes, multipotential hematopoietic stem cells and committed granulocyte/macrophage progenitor cells in the spleen; increased incidence of atypical cell morphology in peripheral blood, bone marrow, and spleen. This study identifies a NOAEL of 9.6 ppm (31 mg/m^3) for slight hematopoietic effects in mice exposed to benzene for 10 weeks and a LOAEL of 302 ppm (966 mg/m^3) for severe hematopoietic toxicity in mice exposed for 26 weeks.

Sprague-Dawley rats (50/sex/group; 12 weeks of age) and CD-1 mice (150/sex/group; 9 weeks of age) were exposed to nominal vapor concentrations of 0, 1, 10, 30, or 300 ppm benzene (99.9% purity) (0, 3, 32, 96, or 959 mg/m^3), 6 hours/day, 5 days/week, for 13 weeks (Ward et al., 1985). Clinical observations and body weight data were normal in both species. High-exposure level rats had leukopenia and significantly ($p<0.05$) decreased femoral marrow cellularity. High-exposure level mice had leukopenia, anemia, thrombocytopenia, and significant increases in MCV, MCH, glycerol lysis time, and incidence and severity of

morphological changes in RBC. Relative testes weights were significantly decreased in high-dose male mice. High-dose mice had histological abnormalities in the thymus (atrophy), bone marrow (myeloid hyperplasia), lymph nodes (lymphoid depletion of mesenteric and mandibular lymph nodes; plasma cell infiltration into mandibular lymph node), spleen (increased incidence of extramedullary hematopoiesis; periarteriolar lymphoid sheath depletion), ovaries (bilateral ovarian cysts), and testes (bilateral atrophy/degeneration; decreases in spermatozoa in the epididymal ducts; increased numbers of abnormal sperm types); similar lesions were observed in the testes and ovaries of mice exposed to concentrations lower than 300 ppm (959 mg/m^3), but the authors did not consider these effects to be biologically significant. The incidence and severity of most benzene effects were greater in male mice than in female mice. This study identifies a NOAEL of 30 ppm (96 mg/m^3) and a LOAEL of 300 ppm (959 mg/m^3) for these effects in rats and mice.

Male C57BL/6 mice were exposed to 0 or 300 ppm benzene (0 or 960 mg/m^3), 6 hours/day, 5 days/week for 9 weeks (Baarson and Snyder, 1991). Blood was withdrawn from the tail vein for differential white blood cell counts and peripheral nucleated red cell counts. Following sacrifice, femurs and the spleen were aseptically removed and placed in sterile culture dishes. Single cell suspensions were made, and the numbers of colony forming unit erythroid (CFU-E) cells and burst forming unit erythroid (BFU-E) cells were counted in control vs. exposed animals. From 1 day after beginning of exposure to benzene until the end of the treatment period, peripheral red blood cell counts were decreased. In addition, the numbers of BFU-E and CFU-E colonies were depressed to less than half the control values, in all exposed animals at days 5 and 60 of exposure. These effects represent an adverse effect on the organism, due to the potential for anemia. In this study, the combined treatment of benzene and ethanol was studied in a second group of animals, and was the thrust of the new information from this group. The exposure levels are far greater in this study than in previous work; a LOAEL of 960 mg/m^3 (HEC=171 mg/m^3) was established for hematotoxic effects.

Similar results were seen in a study where female C57BL/6xDBA/2 F1 hybrid mice were exposed to 0 or 300 ppm benzene (0 or 960 mg/m^3), 6 hours/day, 5 days/week for 6-7 weeks (Vacha et al., 1990). Indices of hematopoiesis were measured in peripheral blood (RBC and WBC count, Hb, Hct, reticulocyte, and leukocyte count), in addition to ^{59}Fe accumulation in the erythropoietic organs (spleen and bone marrow) and in the peripheral RBC's. The distribution of developmental classes of erythroblasts was also determined. This study found that animals became anemic after 6-7 weeks of benzene exposure. The number of erythroblasts in the bone marrow was not different, however exposure to benzene shifted the population to a less mature class of cells. The number of colonies derived from BFU-E and CFU-E were decreased to 70% and 34% of controls, respectively. A LOAEL of 960 mg/m^3 (HEC=171 mg/m^3) was established for hematotoxic effects.

BDF1 mice were exposed to 0, 100, 300, or 900 ppm benzene (0, 320, 960, 2880 mg/m^3) for up to 4 weeks (Seidel et al., 1990). The numbers of hematopoietic progenitor cells, early and late progenitors (BFU-E, CFU-E), and granuloid progenitors (CFU-C) were determined. A group was generated to establish the effect of ethanol (drinking water) on

these effects. This study demonstrated that the number of CFU-E per femur was decreased in a concentration-dependent manner by benzene. This effect was evident at 300 and 900 ppm (960 and 2880 mg/m^3, respectively) concentions, however the effect of the 100 ppm (320 mg/m^3) exposure group was uncertain, as the study focused on the effect of ethanol on benzene toxicity. The LOAEL/NOAEL was thus difficult to determine.

Male Sprague-Dawley rats (40/group) were exposed to vapor concentrations of 0 or 100 ppm benzene (0 or 319 mg/m^3), 6 hours/day/ 5 days/week, for life (American Petroleum Institute, 1983). Blood samples were obtained at 2-4 week intervals throughout the treatment period. The treatment had no adverse effects with respect to mortality rates or body weight gain. Peripheral erythrocyte and lymphocyte counts were depressed at nearly every sampling time in treated rats, but the extent of decrease was not statistically significant at $p<0.05$. Significantly increased incidence of splenic hyperplasia ($p<0.005$) and hemosiderin pigments ($p<0.001$) were observed in benzene-exposed rats. The incidences of normally rare tumors in treated rats were liver (4/40), Zymbal gland (2/40), and chronic myelogenous leukemia (1/40); the authors considered these tumors to be related to the benzene exposure. This study identifies a LOAEL of 100 ppm (319 mg/m^3) for slight hematological effects in rats.

Male AKR/J (50/group) and C57BL/6J mice (40/group) were exposed to vapor concentrations of 0, 100 (319 mg/m^3; AKR mice only), or 300 ppm (958 mg/m^3; C57BL/J mice only) benzene, 6 hours/day, 5 days/week for life (Snyder et al., 1980). The following parameters were used to assess toxicity: clinical signs (observed daily), body weights (measured biweekly), hematology (RBC, WBC, WBC differentials, absolute neutrophil and lymphocyte; measured biweekly in 10 control and 10 treated mice from each strain), and gross and microscopic necropsy (lung, liver, spleen, kidney, and bone marrow). The treatment had no adverse effects with respect to life span, body weight, or incidence of lymphoma in AKR mice. Treated AKR mice had significant (± 2 standard errors) degrees of lymphocytopenia, neutrophilia, erythropenia, and bone marrow hypoplasia ($p<0.05$). Treated C57BL mice had significant (± 2 standard errors) degrees of lymphocytopenia, neutrophilia, erythropenia, morphological changes in peripheral blood cells, and bone marrow and splenic hyperplasia ($p<0.05$). The incidence of hematopoietic neoplasms was significantly ($p<0.05$) increased in C57BL mice, including 6 cases of thymic lymphoma. This study identifies a LOAEL of 100 ppm (319 mg/m^3) for hematopoietic effects in mice.

Pregnant Swiss Webster mice (5/exposure level/progeny age group; initial age 8-12 weeks) were exposed via inhalation to nominal vapor concentrations of 0, 5, 10, or 20 ppm benzene (0, 16, 32, or 64 mg/m^3) for 6 hours/day on gestation days 6-15 (Keller and Snyder, 1988). On gestation day 16 (fetuses), 2 days after birth (neonates), and 6 weeks after birth (adults), progeny (1-2 males and 1-2 females/litter) were sacrificed to determine the amounts and types of hemoglobin produced, and hemopoietic cells in the peripheral blood and hematopoietic organs. No evidence of maternal or non-hematopoietic developmental toxicity was observed in treated mice, and no adverse hematopoietic effects were observed in fetuses. The treatment had no adverse effects in any progeny with respect to peripheral blood levels of RBC, MCH, blasts, dividing granulocytes, lymphocytes, or ratio of hemoglobin A major to hemoglobin A minor. There was a concentration-related decrease in peripheral blood levels

of early nucleated red cells in neonates, significant ($p<0.05$) at all exposure levels. High-exposure level neonates had significantly ($p<0.05$) increased numbers of nondividing granulocytes and decreased numbers of late nucleated red cells in peripheral blood. In high-exposure level neonates, hepatic levels of blasts, dividing granulocytes, non-dividing granulocytes, and lymphocytes were significantly ($p<0.05$) increased and late nucleated red cells were significantly ($p<0.05$) decreased; hepatic levels of blasts were also significantly ($p<0.05$) increased at the low-exposure level in neonates. In adults, there was a concentration-related decrease in early nucleated red cells in bone marrow, significant ($p<0.05$) at the high-exposure level. High-exposure level adults also had significant ($p<0.05$) increases in splenic levels of blasts, dividing granulocytes, and nondividing granulocytes; splenic levels of non-dividing granulocytes were also increased in low-exposure level adults. This study identifies a LOAEL of 5 ppm (16 mg/m^3) for developmental hematopoietic effects in mice.

Pregnant Swiss-Webster mice (5/exposure level/progeny age group; initial age 8-12 weeks) were exposed via inhalation to nominal vapor concentrations of 0, 5, 10, or 20 ppm benzene (0, 16, 32, or 64 mg/m^3) for 6 hours/day on gestation days 6-15 (Keller and Snyder, 1986). On gestation day 16 (fetuses), 2 days after birth (neonates), and 6 weeks after birth (adults), progeny (1-2 males and 1-2 females/litter) were sacrificed for measurement of hematopoietic progenitor cells [colony forming units - erythroid (CFU-E), burst forming units - erythroid (BFU-E), and granulocytic colony forming cells (GC-CFU-C)] from the liver (fetuses and neonates), and bone marrow and spleen (adults). In addition, 10-week old progeny from litters in the control and mid-exposure group were exposed for 2 weeks to 10 ppm (32 mg/m^3) benzene, then sacrificed for measurement of hematopoietic progenitor cells from the bone marrow and spleen. There was no evidence of maternal or non-hematopoietic developmental toxicity in benzene-exposed mice. There was a significant ($p<0.05$) increase in the numbers of erythroid burst forming units from livers of male and female fetuses exposed to the low- and mid-exposure level, respectively. The following significant ($p<0.05$) changes were observed with respect to CFU-E: in fetuses, there were increases in liver CFU-E at the low- and mid-exposure levels and decreases at the high-exposure level; in male neonates, there were increases and decreases in liver CFU-E at the mid-exposure level, and increases at the high-exposure level; in adult mice there were decreases in bone marrow CFU-E and increases in spleen CFU-E in males exposed to 10 ppm (32 mg/m^3) in utero. Liver GM-CFU-C in neonates was significantly ($p<0.05$) decreased at the mid-exposure level (males only) and increased at the high-exposure level. Mice exposed to 10 ppm (32 mg/m^3) benzene in utero and for 2 weeks as adults had significantly ($p<0.05$) decreased bone marrow CFU-E (males only) and splenic GM-CFU-C; mice exposed to air in utero and 10 ppm (32 mg/m^3) benzene for 2 weeks as adults had no changes in bone marrow or splenic CFU-E, but had a significant ($p<0.05$) decrease in splenic GM-CFU-C (females only). The authors concluded that benzene treatment in utero induced hematopoietic alterations in fetuses, persisting until at least 10 weeks after birth. This study identifies a LOAEL of 5 ppm (16 mg/m^3) for developmental hematopoietic effects in mice.

Bred Sprague-Dawley rats (17-20/group; initial body weights 210-223 g) were exposed via inhalation to nominal vapor concentrations of 0, 10, 50, or 500 ppm benzene (0, 32, 160,

and 1600 mg/m^3) for 7 hours/day, on gestation days 6-15, followed by sacrifice on gestation day 20 for determination of developmental abnormalities (Kuna and Kapp, 1981). The treatment had no adverse effects on dams with respect to mortality rate, hematology, or gross necropsy. Body weight gain over gestation days 5-15 was significantly ($p<0.05$) decreased in mid- and high-exposure level dams. Fetal body weight was significantly ($p<0.05$) decreased at the mid- and high-exposure levels and fetal crown-rump length was decreased at the high-exposure level. The number of litters with skeletal and visceral variants was significantly ($p<0.05$) increased at the mid- and high-exposure levels. The skeletal and visceral abnormalities observed included execephaly, angulated ribs, dilated lateral and third ventricles of the brain, forefeet ossification out of sequence, generalized lagging ossification, and decreased numbers of caudals, and metacarpals, metatarsals, and phalanges/foot; the authors considered these abnormalities to be related to the benzene treatment. This study identifies a NOAEL of 10 ppm (32 mg/m^3) and a LOAEL of 50 ppm (160 mg/m^3) for maternal toxicity and developmental effects in rats.

Bred Sprague-Dawley rats (26-31/group) were exposed to nominal vapor concentrations of 0, 10, or 40 ppm benzene (0, 32, or 128 mg/m^3) for 6 hours/day on gestation days 6-15 (Litton Bionetics, 1978). The treatment had no adverse effects on mortality rate, body weight gain, or food consumption in dams. Pregnancy ratio, fetal weight, live litter size, and incidence of variants and malformations were similar in control and treatment groups. Benzene-exposed rats had significantly ($p<0.05$) decreased ratio of live fetuses/implantation site. The number of resorption sites was increased in benzene-exposed rats, but the difference was only significant ($p<0.05$) in the low-exposure group. This study identifies a LOAEL of 10 ppm (32 mg/m^3) for developmental effects in rats.

Female Sprague-Dawley rats (26/group) were exposed to vapor concentrations of 0, 1, 10, 30, or 300 ppm benzene (0, 3, 32, 96, or 958 mg/m^3), 6 hours/day, 5 days/week during premating (10 weeks) and mating periods, then 6 hours/day, 7 days/week, on gestation days 1-20, and lactation days 5-21 (Bio/dynamics, 1980). The following parameters were used to assess toxicity: clinical signs, mortality rate, body weight gain, pregnancy rates, and gestation length in dams; number alive and dead at birth, sex distribution, survival, body weights, organ weights, and gross necropsy in pups. The treatment had no adverse effects with respect to reproduction or maternal toxicity. This study identifies a NOAEL of 300 ppm (958 mg/m^3) for reproductive effects and maternal and developmental toxicity in rats.

Coate et al. (1984) exposed pregnant Sprague-Dawley rats (40/group) to 1, 10, 40, or 100 ppm benzene (3.2, 32, 128, or 320 mg/m^3), 6 hr/day, days 6-15 of gestation. No maternal toxicity or teratogenic effects were noted. There was no deviation from controls in the number of resorptions. There was reduced fetal weight at 100 ppm ($p<0.05$) thus, a LOAEL of 100 ppm (320 mg/m^3) can be identified along with a NOAEL of 40 ppm (128 mg/m^3).

Unovary and Tatrai (1985) exposed pregnant CFLP mice (15/group) and New Zealand rabbits (11-15/group) to 0, 160 or 320 ppm benzene (0, 500 or 1000 mg/m^3), 24 hr/day, during days 6-15 (mice) or 7-20 (rabbits) of gestation. There were no teratogenic effects in

either species. In rabbits, exposure to 320 ppm (1000 mg/m^3) was associated with reduced fetal weight ($p<0.05$) in the presence of reduced maternal body weight gain. The NOAEL and LOAEL for this effect was 160 ppm (500 mg/m^3) and 320 ppm (1000 mg/m^3), respectively. Mice exposed to concentrations ≥160 ppm (500 mg/m^3) had reduced fetal weight, as well as skeletal deformities (maternal weight data not provided). The LOAEL for this effect was 160 ppm (500 mg/m^3).

DERIVATION OF PROVISIONAL CHRONIC INHALATION RfC

Chronic exposure of humans to benzene vapor in the work place resulted in hematological and/or hematopoietic effects at concentrations of 30-218 ppm (96-697 mg/m^3) (Askoy et al., 1971; Fishbeck et al., 1978; Kipen et al., 1989). At lower concentrations (0.01-20 ppm; 0.03-64 mg/m^3), no adverse hematological effects were observed in peripheral blood of humans (Kipen et al., 1989; Collins et al., 1991). However, it is not known whether chronic exposure to low concentrations of benzene affects hematopoiesis in the bone marrow and spleen in humans.

The most sensitive endpoint for long-term exposure to benzene vapor is toxicity to hematopoietic progenitor cells. The lowest LOAEL identified for this effect are at 10 ppm (32 mg/m^3) in mice exposed to benzene subchronically (Baarson et al., 1984). Green et al. (1981a,b) identified NOAELs for damage to hematopoietic progenitor cells at 10 ppm (32 mg/m^3). Ward et al. (1985) established a NOAEL of 30 ppm (96 mg/m^3) for hematological effects, but effects on the progenitor cells were not evaluated. Lifetime studies provide evidence that mice are more sensitive to the long-term effects of benzene than are rats (Snyder et al., 1980; American Petroleum Institute, 1983). Reproductive effects (testicular lesions and ovarian cysts) were observed in mice exposed to 300 ppm (959 mg/m^3) for 13 weeks, but not in mice exposed to 30 ppm (96 mg/m^3) (Ward et al., 1985), or in female rats exposed to 300 ppm (959 mg/m^3) for 17 weeks during premating, mating, gestation, and lactation (Bio/dynamics, 1980). The Keller and Snyder (1988) developmental toxicity study identified a LOAEL of 5 ppm (16 mg/m^3), however the $LOAEL_{HEC}$ of 16 mg/m^3 is higher than the $LOAEL_{HEC}$ of 5.7 mg/m^3 from the Baarson et al. (1984) and Green et al. (1981a,b) subchronic studies.

The Baarson et al. (1984) study was selected as the critical study because the exposure period was longer (25 vs. 10 weeks) at the low dose of 10 ppm (32 mg/m^3) than that of the Green et al. (1981a,b) studies.

a. LOAEL of 10 ppm (32 mg/m^3) from the Baarson et al. (1984) studies was adjusted for intermittent exposure:

$$LOAEL_{ADJ} = 32 \text{ mg/m}^3 \times 6 \text{ hours}/24 \text{ hours} \times 5 \text{ days}/7 \text{ days} = 5.7 \text{ mg/m}^3.$$

b. Derivation of the $LOAEL_{HEC}$:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times L_A/L_H$$

where: L_A = blood:air partition coefficient for benzene in male B6C3F1 mice (12.1) (Gargas et al., 1989)

L_H = blood:air partition coefficient for benzene in humans (8.19) (Gargas et al., 1989)

$LOAEL_{HEC} = 5.7\ mg/m^3 \times 1.0 = 5.7\ mg/m^3$; because the ratio of animal to human blood:air partition coefficients is greater than 1 (1.48), the default ratio of 1 is used (U.S. EPA, 1987).

An uncertainty factor of 1000 was applied to the $LOAEL_{HEC}$ of 5.7 mg/m^3 to yield a provisional RfC of 6×10^{-3} mg/m^3. The uncertainty factor includes 3 for interspecies extrapolation using dosimetric adjustments, 10 for intraspecies variability, 10 for use of a subchronic study, and 3 to extrapolate from a minimal LOAEL.

Confidence in the key study (Baarson et al., 1984) is low. A small number of animals of one sex were used. Green et al. (1981a,b) identified NOAELs at the same dose level for similar endpoints. Confidence in the database is medium. A large number of studies corroborated the hematopoietic effects observed in the Baarson et. al. (1984) study. In addition, testicular lesions were reported by Ward et al. (1985); however, male reproductive performance tests and/or a multigeneration reproduction study were not identified. Reflecting the low confidence in the key study and medium confidence in the database, confidence in this provisional chronic RfC is low.

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Benzene Chronic RfC Principal/Supporting Studies - Inhalation Exposure

Study	Species/ Route	Conc. (ppm/ mg/m^3)	Duration	Critical Effect	NOAEL (mg/m^3)	LOAEL (mg/m^3)	NOAEL[adj] LOAEL[adj] (mg/m^3)	HEC (mg/m^3)
Co-critical Studies:								
Baarson et al. 1984 (chronic)	C57BL/J mice	0/0 10/32	6 h/d 5 d/wk 178 d	Decr. ability of mouse marrow progenitor cells to form colonies	None	32	5.7 L	5.7 L
Green et al. (1981a,b) (acute and chronic)	CD-1 mice	0/0 9.6/31	6 h/d 5 d/wk 10 wk	Hematolo-gical	31	None	5.5 N	5.5 N
		0/0 302/966	26 wk	None	966	173 L	173 L	
Supporting Studies:								
Aksoy et al. 1971	Human	0/0 30/96 218/696	3 m to 17 yr	Hematolo-gical '	None	96	34 L	
Collins et al. 1991	Human	0/0 0.045/0.14	7 yr	None	0.14 (free-standing)	None	0.05 N	

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Study	Species/ Route	Conc. (ppm/ mg/m^3)	Duration	Critical Effect	NOAEL (mg/m^3)	LOAEL (mg/m^3)	NOAEL[adj] LOAEL[adj] (mg/m^3)	HEC (mg/m^3)
Ward et al. 1985 (subchronic)	CD-1 mice	0/0 1/3 10/32 30/96 300/959	6 h/d 5 d/wk 13 wk	Hematological effects (decr. RBC, WBC, platelets, Hb, M/E ratios, and Hct.; Histological effects (91 d postexp): testicular atrophy, abn. sperm, decr. spermatozoa, etc.	96	959	17 N 171 L	17 N 171 L
	Rats			Decr. lymphocyte count, incr. neutrophils; Histological effects: decr. femoral marrow cellularity (at 7 d exposure)	96	959	17 N 171 L	17 N 171 L
Baarson and Snyder 1991 (subchronic)	C57BL/6 male mice	0/0 300/960	6 h/d 5 d/wk 9 wk	Decreased RBC count; decr. ability of mouse marrow progenitor cells to form colonies	None	960	171L	171 L

Study	Species/ Route	Conc. (ppm/ mg/m^3)	Duration	Critical Effect	NOAEL (mg/m^3)	LOAEL (mg/m^3)	NOAEL[adj] LOAEL[adj] (mg/m^3)	HEC (mg/m^3)
Vacha et al. 1990 (subchronic)	C57BL/6x DBA/2 F1 hybrid female mice	0/0 300/960	6 h/d 5 d/wk 6-7 wk	Decreased RBC count; decr. ability of mouse marrow progenitor cells to form colonies	None	960	171 L	171 L
Seidel et al. 1990 (subchronic)	BDF1 mice	0/0 100/320 300/960 900/2880	6 h/d 5 d/wk 4 wk	Decreased ability of mouse marrow progenitor cells to form colonies	Unclear	960	171 L	171 L
Kuna and Kapp 1981 (develop-mental)	Sprague-Dawley rats	0/0 10/32 50/160 500/1600	7 hr/d GD 6-15	Decr. fetal body weight	32	160	32 N 160 L	32 N 160 L
Coate et al. 1984 (develop-mental)	Sprague-Dawley rats	1/3.2 10/32 40/128 100/320	6 hr/d GD 6-15	Decr. fetal body weight	128	320	128 N 320 L	128 L 320 L

Study	Species/ Route	Conc. (ppm/ mg/m^3)	Duration	Critical Effect	NOAEL (mg/m^3)	LOAEL (mg/m^3)	NOAEL[adj] LOAEL[adj] (mg/m^3)	HEC (mg/m^3)
Unovary and Tatrai 1985 (develop-mental)	CFLP mice	0/0 160/500 320/1000	24 hr/d GD 6-15	Decr. fetal body weight	None	500	500 L	500 L
	New Zealand white rabbits		GD 7-20	Decr. fetal body weight	500	1000	500 N 1000 L	500 N 1000 L

Attachment 2

(94-007/04-11-94)

Risk Assessment Issue Paper for:
Derivation of a Provisional Inhalation RfC for
Carbon Tetrachloride (CASRN 56-23-5)

Introduction

Review documents available for carbon tetrachloride include a Health Assessment Document (U.S. EPA, 1984), a Drinking Water Criteria Document (U.S. EPA, 1985), an Updated Health Effects Assessment (U.S. EPA, 1989) and an Updated Toxicological Profile (ATSDR, 1992a). These documents were examined for information pertinent to derivation of an RfC for carbon tetrachloride. Computer searches of TOXLINE (1986-1992), TSCATS, HSDB and RTECS were performed in August, 1992 and screened for relevant information. Updated computer literature searches of TOXLINE (1992-1994), MEDLINE (1992-1994) and RTECS were conducted and screened in March, 1994. The OHEA CARA list (U.S. EPA, 1991, 1993), NTP status reports (NTP, 1994a, 1994b) and ATSDR Toxicological Profile Information Sheet (ATSDR, 1992b) were also consulted.

An inhalation RfC for carbon tetrachloride has not been discussed by the RfD/RfC Work Group (U.S. EPA, 1994a) and is not available on IRIS (U.S. EPA, 1994b) or in the HEAST (U.S. EPA, 1994c). However, ATSDR (1992a) derived an intermediate inhalation MRL of 0.01 ppm for carbon tetrachloride.

Review of Pertinent Literature

No studies were located regarding the subchronic or chronic toxicity of inhaled carbon tetrachloride vapor to humans. In animals, several subchronic inhalation studies were available. In the earliest of these studies, Smyth et al. (1936) exposed groups of 24 guinea pigs (strain not specified) and 24 Wistar-derived rats (mixed sexes of both species) to 50, 100, 200 or 400 ppm (315, 630, 1260 or 2520 mg/m^3) of carbon tetrachloride vapor (>99% pure), 8 hours/day 5 days/week for up to 10.5 months. The guinea pigs in this study received a purely vegetarian diet, but because the authors felt that low calcium in this diet may have affected the toxicity results,
additional groups of 16 guinea pigs fed diets supplemented with calcium were tested at concentrations of 25 ppm (157 mg/m^3), as well as 50, 100 and 200 ppm. In addition to the rats and guinea pigs, groups of 4 monkeys (species and sex not specified) were exposed to 50 or 200 ppm using the same protocol. Use of controls was not described although controls apparently were included in the study. All animals were weighed weekly. Blood counts (all species) and urinalysis (guinea pigs and monkeys) were performed monthly. The fertility of rats and guinea pigs, which were housed in mixed-sex groups and produced litters during the study, was monitored. All animals that survived to scheduled sacrifice (including some

animals which were sacrificed only after recovery periods of varying durations), and most of those dying during the study, were examined for gross pathology. Tissue samples for histopathological examination were taken from the liver, kidney, adrenal gland, spleen, heart, sciatic and optic nerves and ocular muscle. Serum chemistry analyses were performed on some animals as well. No statistical tests were conducted. Guinea pigs of all exposure groups, including those that received diets supplemented with calcium, suffered substantial mortality (≥25-80% among "uninfected" guinea pigs). Mortality in controls was not reported. In contrast, mortality among "uninfected" rats was limited to 2 animals exposed to 400 ppm. No monkeys died during the study. Compared to controls, body weight gain was reported to be markedly reduced among survivors in all groups of guinea pigs. Body weight gain was also reduced by about 30% among rats exposed to 400 ppm. Too few litters were born to guinea pigs during the study to determine if exposure had any effect, but in rats fertility was reduced in the 200 and 400 ppm groups. In guinea pigs, fatty changes in the liver were seen at all dose levels, and cirrhosis developed at ≥50 ppm. In rats, fatty changes were seen at ≥50 ppm and cirrhosis at ≥100 ppm. In monkeys, mild fatty degeneration of the liver was found at both 50 and 200 ppm. Other pathological changes in animals exposed to these concentrations included renal tubular degeneration, degeneration of the adrenal glands (with necrosis in guinea pigs) and damage to the sciatic nerve. This study did not include concentrations low enough to identify a NOAEL for any of the 3 species tested. For guinea pigs, the low concentration of 25 ppm was a FEL that produced substantial mortality. For rats and monkeys, the low concentration of 50 ppm was a LOAEL that produced fatty changes in the liver.

Adams et al. (1952) conducted studies in which Wistar-derived rats (15-25/sex), outbred guinea pigs (5-9/sex), outbred rabbits (1-2/sex) and rhesus monkeys (1-2 of either sex) were exposed to carbon tetrachloride vapor (>99% pure), 7 hours/day and 5 days/week for 6 months at concentrations of 5, 10, 25, 50, 100, 200 or 400 ppm (31, 63, 157, 315, 630, 1260 or 2520 mg/m^3). Matched control groups, both unexposed and air-exposed, were included in these experiments. Animals were observed frequently for appearance and general behavior, and weighed twice weekly. Selected animals were used for hematological analyses periodically throughout the study. Moribund animals and those surviving to scheduled sacrifice were necropsied. The lungs, heart, liver, kidneys, spleen and testes were weighed, and sections from these and 10 other tissues were prepared for histopathological examination. In many cases, terminal blood samples were collected and used for serum chemistry analyses, and part of the liver was frozen and used for lipid analyses. The t-test was used to make statistical comparisons. Guinea pigs were the most sensitive species tested. The primary target of carbon tetrachloride in guinea pigs was the liver. Liver effects progressed from a statistically significant increase in relative liver weight among females at 5 ppm to include slight-to-moderate fatty degeneration and increases in liver total lipid, neutral fat and esterified cholesterol at 10 ppm, and cirrhosis at 25 ppm. Liver effects became progressively more severe at higher concentrations. Growth retardation was first observed at 25 ppm and progressed to rapid loss of weight at 200 ppm. In the kidney, slight tubular degeneration was first observed at 200 ppm and increased kidney weight at 400 ppm. Mortality was increased at ≥100 ppm. A similar progression of effects was seen in rats, with no effects at 5 ppm, mild liver changes at 10 ppm, cirrhosis at 50 ppm and liver necrosis, kidney effects, testicular

atrophy, growth depression and mortality at ≥200 ppm. In rabbits, 10 ppm was without effect, 25 ppm produced mild liver changes, 50 ppm produced moderate liver changes and 100 ppm produced growth depression. Monkeys were the most resistant species tested, with evidence of adverse effects (mild liver lesions and increased liver lipid) only at 100 ppm, the highest concentration tested. This study identified a LOAEL of 5 ppm in guinea pigs and NOAEL and LOAEL values, respectively, of 5 and 10 ppm in rats, 10 and 25 ppm in rabbits and 50 and 100 ppm in monkeys, all based on hepatotoxic effects. The intermediate inhalation MRL calculated by ATSDR (1992a) was based on the occurrence of liver effects in rats in this study, using the same NOAEL and LOAEL values cited here.

Prendergast et al. (1967) exposed groups of 15 Sprague-Dawley or Long-Evans rats, 15 Hartley guinea pigs, 3 New Zealand rabbits, 2 beagle dogs and 3 squirrel monkeys (sexes not specified) to carbon tetrachloride vapor ("highest purity available") either by continuous exposure to 6.1 or 61 mg/m^3 (1 or 10 ppm) for 90 days or intermittent exposure (8 hours/day, 5 days/week) to 515 mg/m^3 (82 ppm) for 6 weeks. The control group consisted of 304 rats, 314 guinea pigs, 48 rabbits, 34 dogs and 57 monkeys. In order to generate the 6.1 mg/m^3 concentration, the researchers found it necessary to dilute the carbon tetrachloride in 61 mg/m^3 of n-octane. Therefore, a vehicle control group exposed to 62 mg/m^3 of n-octane was included in this study. Animals were observed routinely for signs of toxicity and weighed monthly. Blood samples for hematological analysis were taken at the end of the exposure period. Following sacrifice, animals were necropsied and sections of the heart, lung, liver, spleen and kidney were taken for histopathological examination. Serum chemistry and liver lipid analyses were performed on some animals. No statistical tests were conducted. Intermittent exposure to 515 mg/m^3 resulted in the death of 3 guinea pigs and 1 monkey. Body weight gain was reduced in all species, and all species except rats actually lost weight during the study. Mottled livers were seen in all species except dogs. Histopathological examination of the liver revealed fatty changes that decreased in severity from guinea pigs to rats to rabbits to dogs to monkeys. Liver lipid content of guinea pigs was increased about 3 fold compared to controls. The only other effect noted was interstitial inflammation in the lungs of all species. Continuous exposure to 61 mg/m^3 resulted in the deaths of 3/15 guinea pigs. Body weight gain was depressed in all species and monkeys appeared visibly emaciated. Gross examination showed the presence of enlarged/discolored livers in all species except dogs. Microscopic examination revealed fatty changes in the liver that were most prominent in rats and guinea pigs, but present in the other species as well. Lung effects were not reported in this group. Continuous exposure to 6.1 mg/m^3 produced reduced growth in all species, although the only histopathological findings were nonspecific inflammatory changes in the liver, kidney, heart and lungs. No effects were noted in the n-octane control group. The results of this study suggest a LOAEL of 6.1 mg/m^3 for all species tested based on reduced growth, but the study was limited by failure to compare growth to controls using statistical tests. It is not clear whether inflammatory changes observed in the lungs of some exposed animals occurred in controls as well.

More recent inhalation studies of carbon tetrachloride toxicity were performed for purposes other than determination of NOAEL and LOAEL values and were not useful for risk assessment for various reasons, including use of only a single exposure level (usually a high

one), brief daily exposure periods and short study durations (Bogers et al., 1987; Hall et al., 1991; Plummer et al., 1990; Sakata et al., 1987; Vazquez et al., 1990).

As noted above, Adams et al. (1952) found testicular degeneration in rats exposed to 200 or 400 ppm (1260 or 2520 mg/m^3) of carbon tetrachloride vapor 7 hours/day, 5 days/week for 6 months. Testicular degeneration was also reported in rats given repeated i.p. doses of 1.5 ml/kg (Chatterjee, 1966; Kalla and Bansal, 1975). Therefore, there is reason to suspect that carbon tetrachloride might affect reproductive performance, and in fact, Smyth et al. (1936) found that fertility was reduced in rats exposed to 200 or 400 ppm (1260 or 2520 mg/m^3) of carbon tetrachloride vapor 8 hours/day 5 days/week for up to 10.5 months. However, reproduction was only studied incidentally in this experiment. Reproductive performance was also monitored in an oral study in which rats of an unspecified strain (18/sex/group) were fed for up to 2 years on experimental diets that had been fumigated with carbon tetrachloride for 48 hours (Alumot et al., 1976). The initial concentrations of carbon tetrachloride in the food were 0, 80 and 200 ppm. The fumigated diet was stored in sealed containers and feeding time was restricted to a single hour during the day and two hours at night so as to prevent excessive volatilization of carbon tetrachloride from the food. The investigators calculated that 60-70% of the carbon tetrachloride present in the food initially was actually present in food consumed by the rats. Based on this estimate and reference values for rat body weight (male = 0.38 kg, female = 0.229 kg) and food consumption (male = 0.03 kg/day, female = 0.021 kg/day) from U.S. EPA (1987), it was calculated that approximate carbon tetrachloride doses of 0, 4 and 10 mg/kg-day and 0, 5 and 12 mg/kg-day were delivered to males and females, respectively. The animals were housed in groups of 6 and segregated by sex for the first 6 weeks of the experiment. At this time the females, now 3 months old, were mated with untreated males. Mating was repeated approximately every 2 months, but using treated males rather than controls. For mating, each male was housed with two females for a 10-day period, during which the animals received the control diet. After the mating period, the animals were returned to their original cages. Females were weighed two times a week, and were transferred to individual cages upon gaining 60 g. Litter size and weight were recorded at parturition and again 10 days later when the dams were returned to their original cages. Treatment with carbon tetrachloride had no effect on the reproductive parameters monitored (male and female fertility, litter size, and pup mortality and body weight at birth and weaning). There was widespread occurrence of chronic respiratory disease in animals from all groups after 14 months, but this probably did not affect these results since most reproductive activity took place during the first year of the study (only 7 successful matings occurred during the second year). Treatment-related parental toxicity was not reported, but only parental body weight was monitored concurrently with the reproductive part of the study. No evidence of liver toxicity was found by serum analyses or biochemical tests at the end of the study. In conclusion, this study found no evidence of reproductive or maternal effects at doses of 10-12 mg/kg-day in the feed.

The developmental effects of carbon tetrachloride have been studied following inhalation exposure. Groups of 22-23 pregnant female Sprague-Dawley rats were exposed to concentrations of 0, 334 or 1004 ppm (0, 2101 or 6316 mg/m^3) for 7 hours per day on gestation days 6-15 (Schwetz et al., 1974). Exposures to the two different dose levels were

not performed concurrently, so two separate control groups were used. Data from the two control groups were combined except where they differed significantly (e.g., incidence of delayed ossification of sternebrae). The rats were observed daily throughout pregnancy. Food intake was monitored every other day during the experiment, and body weight was determined on days 6, 13 and 21 of gestation. Following sacrifice on gestation day 21, the number and uterine position of live, dead and resorbed fetuses were recorded. The fetuses were weighed, measured, and examined for external anomalies. Half of the fetuses in each litter were prepared so as to enable detection of soft tissue anomalies upon subsequent examination, and the remainder were prepared and examined for skeletal abnormalities. The litter was considered the unit of treatment and observation when comparing the results from the different exposure groups. Nonpregnant female rats were exposed simultaneously with the pregnant rats in order to monitor effects on the liver. Serum GPT was determined in these rats throughout exposure and some were sacrificed for gross examination of the liver at the end of the exposure period. The remainder were sacrificed 6 days later (corresponding to the end of gestation in the pregnant rats) for GPT analysis, gross examination of the liver, and determination of liver weight. Significant reductions in fetal body weight and crown-rump length were found in both treated groups. The incidence of delayed ossification of the sternebrae was significantly elevated in the high-dose group compared to the concurrent control, but not compared to the low-dose group or its concurrent control. No other effects on the fetus were reported. Maternal toxicity was also observed in both dose groups. Food consumption and body weight were significantly reduced compared to controls, and hepatotoxicity was indicated by significantly elevated serum GPT, gross changes in liver appearance, and significantly increased liver weight. This study, therefore, detected both maternal and developmental toxicity at 334 ppm.

Several studies of the developmental toxicity of carbon tetrachloride following oral exposure have recently been conducted. Hamlin et al. (1993) treated pregnant female B6D2F1 mice with 0, 82.6 or 826 mg/kg of carbon tetrachloride by gavage in corn oil on days 1-5 of gestation. In this strain, days 1-5 of gestation are characterized by sequential cleavage of the fertilized oocyte to generate a hatched blastocyte, with implantation occurring on day 5 and organogenesis occurring subsequently. Therefore, dosing in this study was limited to the pre-implantation period. A total of 31 pregnant females were included in the experiment, with a minimum of 8 in each dose group (actual group sizes were not reported). Dams were allowed to give birth; litter size was recorded and neonates were weighed, measured for crown-rump length and checked for obvious birth defects. During lactation, the pups were weighed and measured for crown-rump length weekly. Lower incisor eruption and eye opening were assessed in all pups on postpartum days 11 and 15, respectively. Pups were weaned on postpartum day 22 and sacrificed. Dams were weighed weekly during pregnancy and on postpartum day 22 just prior to sacrifice. The liver and kidneys from the dams were removed and weighed. Tissue samples were collected, but not examined for histopathology in time for this report. Treatment with carbon tetrachloride had no effect on dam body weight during pregnancy or on absolute or relative liver or kidney weight at sacrifice. Treatment also had no effect on litter size, pup size at birth, the timing of developmental milestones (incisor eruption and eye opening) or pup growth through weaning (a statistically significant difference in body weight between high-dose pups and controls on day 15 postpartum was not

considered to be biologically significant by the researchers since crown-rump length was not affected and no other body weight differences were found). No stillbirths or malformations were observed. The value of this study is limited because the results were not well reported and no data were shown.

In two recent abstracts, Narotsky et al. (1992, 1993) report that carbon tetrachloride induced full-litter resorption in F-344 rats when pregnant rats were treated with 150 mg/kg by gavage in corn oil during organogenesis (GD 6-15 or GD 6-7). This dose level also caused maternal weight loss on GD 6-8. Resorption rates in surviving litters were unaffected by treatment.

Derivation of RfC

A provisional RfC for carbon tetrachloride can be derived from the study by Adams et al. (1952). This study delineated the progression of toxic effects associated with carbon tetrachloride inhalation and identified NOAEL/LOAEL values in four species. Guinea pigs were the most sensitive species tested, with a LOAEL of 5 ppm (31 mg/m^3), the lowest concentration included in the study. Designation of 31 mg/m^3 as a LOAEL for guinea pigs was based on a statistically significant increase in relative liver weight in females exposed to this concentration. Although increased liver weight does not necessarily indicate a toxic effect, in this case there is a clear progression of liver effects from increased liver weight to fatty changes to cirrhosis with increasing exposure concentration. Therefore, increased liver weight in this case was considered to be an early manifestation of carbon tetrachloride hepatotoxicity and 31 mg/m^3 was taken to be a LOAEL. It should be noted, however, that ATSDR (1992a) considered 31 mg/m^3 to be a NOAEL in guinea pigs and chose to use rats as the basis for the intermediate inhalation MRL.

To calculate the RfC, the LOAEL of 31 mg/m^3 is multiplied by the exposure period (7 hr/24 hr)(5 d/7 d) to obtain a duration-adjusted $LOAEL_{ADJ}$ of 6.5 mg/m^3. The human equivalent concentration (HEC) for an extrarespiratory effect produced by a vapor is calculated by multiplying the $LOAEL_{ADJ}$ by the ratio of blood:gas partition coefficients in animals and humans (L_A/L_H). Since a value for L_A is not available for guinea pigs, a default value of 1 is used for L_A/L_H and the $LOAEL_{HEC}$ becomes 6.5 mg/m^3. An uncertainty factor (UF) of 3000 was calculated from factors of 3 for use of a minimal LOAEL; 3 for interspecies extrapolation using a dosimetric adjustment; 3 for lack of a complete database including adequate study of respiratory, reproductive and developmental endpoints; 10 for use of a subchronic study and 10 to protect sensitive subpopulations. Dividing the $LOAEL_{HEC}$ of 6.5 mg/m^3 by the UF of 3000 produces a provisional RfC of 2E-3 mg/m^3 for carbon tetrachloride.

A provisional RfC of 2E-3 mg/m^3 was calculated for carbon tetrachloride based on hepatotoxicity in guinea pigs inhalation-exposed for 6 months by Adams et al. (1952). Confidence in the principal study is low because group sizes were small (5-9 guinea pigs/sex/group) and data regarding the incidence of histopathological lesions were not

provided. Confidence in the data base is low because no toxicity studies of chronic duration were available and effects on the respiratory tract, reproduction and development were not adequately studied. Low confidence in the RfC follows.

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Attachment 3

(93-20/04-05-93)

Risk Assessment Issue Paper for:
Toxicity and Carcinogenicity of Chloroethane (Ethyl Chloride)
(CASRN 75-00-3)

INHALATION RfC

An inhalation reference concentration for chloroethane is available on IRIS.

PROVISIONAL ORAL RfD

The following sources were checked for pertinent review documents and information: IRIS (U.S. EPA, 1994a), HEAST (U.S. EPA, 1994b), RfD/RfC and CRAVE Work Group Status Reports (U.S. EPA, 1994c,d), OHEA/CARA list (U.S. EPA, 1991,1993), Drinking Water Regulations and Health Advisories list (U.S. EPA, 1994e), and NTP Status Reports (NTP, 1993a,b). The following computer searches, performed in March 1993, were screened to identify additional pertinent studies not discussed in review documents: TOXLINE (oral toxicity and cancer strategies from 1981-1993, inhalation toxicity and cancer strategy from 1965-1993), CANCERLINE (1981-1993), RTECS and HSDB.

The health effects associated with exposure to chloroethane have been reviewed recently by the U.S. EPA (1988a) and the ATSDR (1989). These reviews cited no toxicological or pharmacokinetic data for oral exposures to chloroethane, but sufficient information is available in both reviews for the derivation of an inhalation RfC. The IRIS Supportive Documentation (U.S. EPA, 1988b) explains that adverse effects from one route of exposure may be assumed to be relevant to another route, unless convincing evidence exists to the contrary. However, the observed effect should not be the potential "portal-of-entry" effects. Therefore, in the absence of toxicological and pharmacokinetic data for oral exposure to chloroethane, derivation of a provisional oral RfD based upon inhalation data appears to be appropriate.

Please be aware that the contention that no oral data are available is based upon reviews of the two chloroethane documents cited above, in addition to reviews of drafts of the Reportable Quantity Document for Chloroethane (U.S. EPA, 1983) and the Drinking Water Health Advisory for Chloroethane (U.S. EPA, 1986).

An inhalation RfC for chloroethane (ethyl chloride) was verified in 1990 and is available on IRIS (U.S. EPA, 1994a). The principal study for the RfC was a developmental inhalation study conducted with pregnant CF-1 mice (Scortichini et al., 1986). The study identified a NOAEL (1504 ppm [4.0 g/cu. m] for 6 hours per day on days 6 through 15 of gestation) and a LOAEL (4946 ppm [13 g/cu.m] with the same protocol) for delayed fetal ossification. In deriving the RfC from the NOAEL, duration adjustments were not made,

because the noted effects were developmental. To derive a NOAEL(HEC) from the mouse NOAEL, the attainment of periodicity was assumed. A default value of 1 was used for the ratio of the mouse to human blood:gas partition coefficients, because, although the coefficient for humans is known, that for mice is unknown. The NOAEL(HEC) (4.0 g/cu.m) was divided by an uncertainty factor of 300 (3 for interspecies extrapolation when dosimetric adjstment of inhaled concentrtion is used, 10 for intraspecies variability, 10 for data base deficiencies because of the lack of a multigeneration study and definitive developmental toxicity studies) to obtain an inhalation RfC of 1E+1 mg/cu.m.

Additional studies considered included NTP (1989) subchronic and chronic mouse and rat bioassays that identified free-standing NOAELs for nonneoplastic histological lesions and body weight changes (subchronic NOAEL: 19,000 ppm [50.1 g/cu.m] 6 hours per day, 5 days per week for 13 weeks; chronic NOAEL: 15,000 ppm [39.6 g/cu. m] 6 hours per day, 5 days per week for 102 weeks [rats] or 100 weeks [mice]). Confidence in the principal study was low because it did not establish a firm concentration-response relationship with an adverse effect and did not include an exposure level that was maternotoxic. Confidence in the data base was medium because of the lack of a multigeneration reproductive study in a second species. Medium confidence in the RfC follows.

In consideration of the new inhalation methodology
presented in the Interim Methods for the Development of Inhalation Doses (U.S. EPA, 1989), a reasonable method for deriving an oral RfD from an animal inhalation NOAEL would involve derivation of the NOAEL(HEC) from the duration-adjusted animal NOAEL, followed by derivation of an estimated oral human equivalent NOAEL [NOAEL(OHE)] using the following equation:

$$NOAEL(OHE) = NOAEL(HEC) \times RAF \times IR(human) \times (BWH)^{-1},$$

where RAF = 7
IR(human) = human inhalation rate (20 cu.m/day), and
BWH = human body weight (70 kg).

It is uncertain if application of an absorption factor is warranted in this method, because of the application of the ratio of the blood:gas coefficients in the derivation of the NOAEL(HEC). For the purposes of this exercise, an absorption ratio of 1 has been applied; oral and inhalation absorption have been assumed to be equal.

For the case of chloroethane and the NOAEL from the Scortichini et al. (1986) study, the NOAEL(HEC) of 4 g/cu.m would be multiplied as noted above to obtain a NOAEL(OHE) of 1.143 g/kg/day. Division by an uncertainty factor of 3,000 (300 as discussed for the inhalation RfC and 10 for the route-to-route extrapolation) obtains a provisional oral RfD of 4E-1 mg/kg/day. Low confidence would be associated with this RfD because, although confidence in the principal study is medium, confidence in the data base is low due to lack of toxicological data for oral exposure and lack of a multigeneration reproductive study and a developmental study in a second species.

In summary, this memo discusses the derivation of a provisional oral RfD for chloroethane from animal inhalation data. A route-to-route extrapolation appears to be appropriate, because inhalation data are available, but no oral toxicological data are available. The method described is consistent with the current Interim Methods for Inhalation RfDs (U.S. EPA 1989), but to our knowledge has not been used in an EPA risk assessment. The method yields a numerical value of 4E-1 mg/kg/day. This method employs an uncertainty factor of 10 for route-to-route extrapolation. We are not aware that application of an uncertainty factor for such extrapolation has been conducted before, but we feel that the lack of oral data warrants such an uncertainty factor. You may want to modify this part of the risk assessment.

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PROVISIONAL ORAL SLOPE FACTOR

The carcinogenic assessment for chloroethane has recently been reevaluated. A final decision was not made regarding a weight-of-evidence classification for chloroethane (Group C or Group B2), however, the issues involved in making such a decision are outlined below.

ECAO-Cincinnati has been working on a quantitative carcinogenicity assessment for chloroethane and other chlorinated ethanes that may incorporate pharmacokinetic modeling. However, this effort is not yet completed and is not available at this time.

The following sources were checked for pertinent review documents and information: IRIS (U.S. EPA, 1994a), HEAST (U.S. EPA, 1994b), RfD/RfC and CRAVE Work Group Status Reports (U.S. EPA 1994c,d), OHEA/CARA list (U.S. EPA, 1993), Drinking Water Regulations and Health Advisories list (U.S. EPA, 1994e), and NTP Status Reports (NTP, 1993a,b). These documents include: the ATSDR Toxicological Profile for Chloroethane (ATSDR, 1989), an RQ document (U.S. EPA, 1983), a Drinking Water Health Advisory (U.S. EPA, 1986a), and a TIER I document (U.S. EPA, 1988). The following computer searches, performed in May 1991 and updated in March 1993, were screened to identify

additional pertinent studies not discussed in review documents: TOXLINE (inhalation toxicity and cancer stratagey from 1965-1993, oral toxicity and cancer strategies from 1981-1993), CANCERLINE (1981-1993), MEDLINE 1989-1991, RTECS and HSDB.

WEIGHT-OF-EVIDENCE CLASSIFICATION

Classification -- Group B2, probable human carcinogen classification may be appropriate.

Basis -- There appear to be no human data available, and the available animal data are restricted to a 2-year inhalation NTP bioassay in rats and mice. NTP concluded that clear evidence of carcinogenicity was presented for female mice displaying uncommon carcinomas of the uterus and liver tumors. Data for male mice were considered by the investigators to be inadequate to assess carcinogenic activity due to decreased survival not related to carcinogenic effects, although increased incidence of alveolar/bronchiolar tumors were observed in exposed male mice. NTP reported that equivocal evidence was found for male and female rats displaying skin neoplasms and uncommon malignant astrocytomas of the brain, respectively.

HUMAN CARCINOGENICITY DATA -- Data regarding the carcinogenicity of chloroethane in humans were not located in the available literature.

ANIMAL CARCINOGENICITY DATA -- Data regarding the carcinogenicity of chloroethane in animals are restricted to a report on two-year inhalation studies in B6C3F1 mice and F344/N rats (NTP, 1989). For each species, groups of 50 animals of each sex were exposed to chloroethane concentrations of 0 (inhalation chamber controls) or 15,000 ppm 6 hours per day, 5 days per week for 102 weeks (rats) or 100 weeks (mice). In a preliminary study of the same two species, groups of 10 animals of each sex were exposed to chloroethane concentrations of 0, 2,500, 5,000, 10,000 or 19,000 ppm 6 hours/day, 5 days/week for 13 weeks. No histopathological effects or increased mortality associated with exposure were noted in either species in the 13-week studies, but the final mean body weights of all exposed groups were lower than those of the controls. The largest reduction in body weight was observed in male mice exposed to the highest concentration; mean body weights were 8% lower than that of control males. Even though the preliminary study did not clearly define a MTD for chloroethane, the authors apparently chose the 15,000 ppm level for the 2-year study because of concerns about the potential flammability and explosion hazard of higher concentrations.

No significant differences in survival were noted between exposed and control groups of rats of either sex, but survival of exposed and control male rats was unusually low at the end of the study. The authors reported that unusually high incidences of mononuclear cell leukemia in both control and exposed groups of male rats may have contributed to the high mortality. The authors also reported that survival for all groups was sufficient through weeks 90 and 95 to evaluate carcinogenicity. At the end of the study (102 weeks), survival for male rats was 16/50 (controls) and 8/50 (exposed) and for female rats was 31/50 (controls) and

22/50 (exposed); however, at 90 weeks, survival was 37/50 (control) and 31/50 (exposed) for respective male groups and 43/50 (control) and 33/50 (exposed) for females. Mean body weights of exposed male rats were 4%-8% lower than those of controls after week 33 and in exposed female rats body weights ranged from 5-13% lower than controls after week 11.

Three exposed female rats displayed uncommon astrocytomas (malignant glial cell tumors of the brain). The authors reported that although the overall incidence of malignant glial cell tumors (3/50) was not statistically significantly different from the concurrent controls (0/50), it was statistically significantly increased ($P<0.05$) relative to incidences for previous chamber control groups at the study laboratory (1/297) or for untreated control female F344/N rats from previous NTP studies (23/1,969 = 1%). Primary tumors of glial cell origin were also observed in male rats. One control male had a malignant oligodendroglioma. A benign oligodendroglioma and a malignant astrocytoma were observed in two exposed males.

Five exposed male rats had epithelial tumors of several types with similar characteristics (trichoepithelioma, sebaceous gland adenoma, and basal cell carcinoma). The combined overall incidence (5/50) was not significantly different from the concurrent control incidence (0/50), but statistical significance ($P<0.05$) could be demonstrated when comparisons were made to historical incidences in chamber controls at the study laboratory (2/300) or in untreated controls in NTP studies (30/1,936 = 1.5%).

The authors concluded that the study provided equivocal evidence of carcinogenic activity in both male and female F344/N rats, because although comparisons with concurrent controls indicated no carcinogenic effect, comparisons with historical controls indicated a carcinogenic effect.

Survival of exposed mice was significantly lower than that of control mice; statistical significance for reduced survival was demonstrated for exposed male mice after day 330 and for exposed female mice after day 574. All surviving mice were sacrificed at 100 weeks. Mean body weights of exposed male mice were up to 13% higher than control male mice. Mean body weights for exposed and female mice were generally similar throughout the study.

Decreased survivability in exposed male mice was not related to tumor occurrences. The authors noted that greater than normal incidences of nonneoplastic urogenital lesions were observed in both control and exposed male mice and that this occurrence may have contributed to the reduced survival. The overall incidences of alveolar/bronchiolar adenomas (8/48) and of alveolar/bronchiolar adenomas and carcinomas (combined) (10/48) were statistically significantly greater ($P<0.05$) than respective incidences for control male mice (3/50 and 5/50). The authors, however, considered the study of male B6C3F1 mice inadequate to evaluate carcinogenic activity because of the reduced survival.

Most of the early mortalities in exposed female mice were associated with carcinomas of the uterus. The overall incidence of uterine carcinomas (all of endometrial gland origin) in exposed female mice (43/50) was greater than that of the concurrent controls (0/49). Uterine

carcinomas were first noted on day 469 of the study. The tumors were highly malignant, and, in 34 animals, metastasized to other organs. Exposed female mice also displayed statistically significantly higher ($P<0.05$) overall incidences of hepatocellular carcinomas (7/48) and hepatocellular carcinomas and adenomas (combined) (8/48) compared to respective incidences in control female mice (3/49 and 3/49). The authors concluded that there was clear evidence of carcinogenic activity in female B6C3F1 mice.

SUPPORTING DATA FOR CARCINOGENICITY -- Two reports provided evidence for the mutagenicity of chloroethane in the closed-desiccator Salmonella typhimurium test for reverse mutations. Riccio et al. (1983) observed mutations in strains TA98, TA100, TA1535 and TA1537 in both the presence and absence of metabolic activation. NTP (1989) observed mutagenic activity in strain TA1535 with or without activation and in strain TA100 only with activation, but no mutagenic activity was observed in strain TA98 with or without activation.

ATSDR (1989) reported that genotoxic activity was not observed in micronucleus tests on bone marrow samples from mice exposed to chloroethane and in cell transformation assays on mouse BALB/c-3T3 cells.

Chloroethane is structurally related to 1,1-dichloroethane, a possible human carcinogen, and to 1,2-dichloroethane and dichloromethane, both of which are probable human carcinogens. (The EPA carcinogen assessments for these related compounds are on IRIS) (U.S. EPA, 1994a).

QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

A provisional q1* for chloroethane is derived below from the NTP (1989) inhalation bioassay data. It should be emphasized that there are uncertainties associated with this value due to the inclusion of only a single high exposure level in the study and the necessity of making the assumption that the carcinogenic effects of chloroethane are not specific for the inhalation route.

The occurrence of uterine carcinomas in female mice was the most dramatic carcinogenic response in the NTP bioassay and therefore appears to provide the most appropriate basis for the derivation of a provisional oral q1*. Statistical adjustments for decreased survival in exposed female mice could be made in a comprehensive quantitative analysis, but, due to time constraints, these adjustments will not be made in the derivation herein. The incidences for the control (0/49) and exposed (43/50) groups were fit to a linearized, multistage model (Global 86). Calculations were based on extra risk. The daily dose for the exposed group of female mice (adjusted for the intermittent experimental exposure protocol) was estimated as follows:

$$\text{Dose} = (39{,}582.8\ \text{mg/m}^3)\ (6\text{h}/24\text{h} \times 5\text{d}/7\text{d})\ (0.052\ \text{m}^3/\text{d})\ (0.031\ \text{kg})^{-1}$$

Dose = 11,856.6 mg/kg/day

where:

39,582.8 mg/m^3 = Exposure conc. = 15,000 ppm x 64.52/24.45, assuming 25 C and 760 mm Hg;

0.031 kg = time-weighted average body weight for female mice estimated from data in the NTP (1989) report;

0.052 m^3/day = inhalation rate (IR) for mice which was estimated using the following equation as described in U.S. EPA (1987):

$$IR = 1.99\ [\text{body weight}]^{1.0496}.$$

The Global 86 model estimated the q1* for mice to be 2.21E-4 per (mg/kg)/day. A provisional human q1* was derived by multiplying the mouse q1* by the cube root of the ratio of the reference human body weight (70 kg) to the animal body weight (0.031 kg), and then by the cube of the lifespan of the animal (100 weeks) to the duration of the experiment as described in the following equation:

Human q1* = 2.21E-4 per (mg/kg)/day x $[70\ kg/0.031\ kg]^{1/3}$ x $[100/100]^3$

Human q1* = 2.21E-4 per (mg/kg)/day) x [13.12] x [1]

Human q1* = 2.9E-3 per (mg/kg)/day).

In summary, a provisional oral slope factor of 2.9E-3 per (mg/kg)/day has been derived for chloroethane based upon incidence data for uterine carcinomas in female B6C3F1 mice exposed to inhaled chloroethane.

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U.S. EPA. 1994e. Drinking Water Regulations and Health Advisories. Office of Water, Washington, D.C. May 1994.

Attachment 4 **(93-22/04-05-93)**

Risk Assessment Issue Paper for:
Derivation of a Provisional Inhalation RfC for
1,2-Dichloroethane (CASRN 107-06-2)

The literature searches conducted on 1,2-dichloroethane onsisted of the following: TOXLINE (1988-1991, inhalation) and RTECS, TSCATS, TOXLIT (1989-1991), and HSDB. In March 1993, update searches were performed on TOXLINE (1991-1993, inhalation and oral) and TSCATS. The following sources were checked for pertinent review documents and information: IRIS (U.S. EPA, 1994a), HEAST (U.S. EPA, 1994b), RfD/RfC and CRAVE Work Group Status Report (U.S. EPA, 1994c,d), OHEA/CARA list (U.S. EPA, 1993), and NTP Status Reports (NTP, 1993a,b). There is an ATSDR Toxicological Profile for 1,2-Dichloroethane (ATSDR, 1989) and the relevant U.S. EPA documents are a 1985 Health Assessment Document for 1,2-Dichloroethane (Ethylene Dichioride), a 1985 Health and Environmental Effects Profile for Dichloroethanes, a 1984 Health Effects Assessment for 1,2-Dichloroethane, and a 1983 Reportable Quantity Document for 1,2-Dichloroethane.

1. Review of available inhalation pharmacokinetics and toxicity information relevant to long-term risk assessment.

A. Absorption and elimination of inhaled 1,2-dichloroethane: 1,2-Dichloroethane is readily absorbed through the lungs following inhalation exposure. Gargas et al. (1989) estimated in vitro blood:air partition coefficients of 19.5 and 30.4 for humans and rats, respectively. In rats, blood 1,2-dichloroethane levels reach steady state within 2-3 hours after the onset of a 6-hour exposure to 50-250 ppm (Reitz et al., 1980; Spreafico et al., 1980). The absorption of 1,2-dichloroethane does not appear to be linear. 1,2-Dichloroethane is lipophilic and is distributed throughout the body (U.S. EPA, 1985a). Un-metabolized 1,2-dichloroethane is excreted through the lungs, while metabolites are predominantly excreted in the urine with some exhaled as CO_2 (U.S. EPA, 1985a). The half-life of whole body elimination is 13-35 minutes after a 5-6 hour inhalation exposure in the rat (Spreafico et al., 1980; Reitz et al., 1980), and 6-8 hours after occupational exposure in man (Urusova, 1953).

B. Repeated exposures of humans: Quantitative data pertinent to the effects of repeated inhalation of 1,2-dichloroethane by humans are limited, and derive chiefly from foreign reports lacking controls and providing inadequate information about duration of exposure and/or the number of subjects exposed (U.S. EPA, 1985a). Case reports of repeated exposures to unknown concentrations describe nervousness, irritability, tremors, depressed reflexes, and irritation of the skin and mucous membranes in exposed workers (U.S. EPA, 1985a). The human data suggest that the LOAEL is in the range of 10-37 ppm; a NOAEL has not been identified. The primary targets of toxicity are the central nervous system, gastrointestinal tract, liver and mucous membranes, including the eyes.

Workers exposed to levels of 1,2-dichloroethane ranging from 60 to 200 ppm reported eye irritation and lacrimation, dryness of the mouth, gastrointestinal disturbances (nausea,

vomiting, loss of appetite), dizziness, and fatigue (Cetnarowicz, 1959). For the most part, these effects were not reported in workers exposed to 10-37 ppm 1,2-dichloroethane. An increased incidence of liver effects (tenderness with enlargement, epigastric pain, altered liver function tests) and gastrointestinal effects (gastritis, pyloric spasms) were also reported in the workers (range of exposures 10-200 ppm) (Cetnarowicz, 1959).

In a group of 100 factory workers exposed to ≤ 25 ppm 1,2-dichloroethane for 6 months to 5 years, heightened lability of the autonomic nervous system, muscular torus, bradycardia, increased hidrosis, and increased frequency of fatigue, irritability, and sleeplessness were reported (Rosenbaum, 1947).

Kozik (1957) reported the results of a study of Russian aircraft industry employees exposed to 1,2-dichloroethane. In this study, morbidity and temporary loss of working capacity was examined during the period of 1951 to 1955. No information on the length of employment or the duration of exposure was reported. For 70-75% of the workshift, the ambient concentration of 1,2-dichloroethane was ≤ 0.05 mg/l (12 ppm), for the remaining 25-30% the levels were 0.08-0.15 mg/l (20-37 ppm). Upon reviewing the data on the ambient air concentrations, NIOSH (1976) concluded that the TWA concentration in the breathing zone was 10-15 ppm. The incidences of morbidity due to acute gastrointestinal disorders, neuritis and radiculitis, and other diseases was higher in the 1,2-dichloroethane exposed workers than other workers (statistical analysis of the data was not presented). A group of 83 1,2-dichloroethane exposed workers received a medical examination. In this group, 19 workers had liver and gall bladder diseases, 13 had neuritis, 11 exhibited autonomic hypotension, and 10 had goiter and hyperthyroidism. Control data were not reported. The authors noted that diseases of the muscles, tendons, and ganglia were considered to be associated with the many repetitive motions the workers had to make when applying the glue. This study has several limitations including the lack of statistical analysis of morbidity data, lack of medical examination of non-exposed workers, examination a limited number of toxicity endpoints, and the lack of control of potentially confounding factors such as alcohol intake.

C. Repeated exposures of animals: The animal data support the findings of the human occupational exposure studies. The primary targets of toxicity include the liver, kidney, and myocardium. The animal data suggest that the concentration-response curve for 1,2-dichloroethane is steep. Exposure levels of > 150 ppm are fatal to rats, mice, guinea pigs, rabbits and monkeys. However, no signs of histopathological damage has been reported in several animal species exposed to 100 ppm (Heppel et al., 1946; Spencer et al., 1951; Hofmann et al., 1971). Alterations of clinical chemistry parameters suggest that exposure levels of 50 ppm or greater may be toxic to the liver and/or kidney (Spreafico et al., 1980). It should be noted that none of the animal studies examined the tracheobronchial or nasal regions of the respiratory tract.

Heppel et al. (1946) exposed several species, including dogs, cats, guinea pigs, rabbits, mice, and rats to 100, 200, 400, or 1,000 ppm (reported analytical values of 0.42, 0.73, 1.54, or 3.9 mg/L) commercial 1,2-dichloroethane for 7 hours/day, 5 days/week. Not all species

were exposed to all concentrations; the duration varied with the exposure level and species; and group size and sex ratio were variable. The exposure levels were not concurrent. Each exposure level was accompanied by control animals, but not all exposed species were represented by controls at each level. Increased mortality was observed in the animals exposed to ≥ 200 ppm. Pulmonary congestion and fatty degeneration of the liver, kidney, and myocardium were also observed in animals exposed to these concentrations. No compound related mortalities were observed at the 100 ppm exposure level; histopathological examinations of the liver, heart, lungs, kidneys, adrenal glands, and spleens of exposed rats and guinea pigs exposed to 100 ppm were unremarkable. No exposure related effect on hematological parameters were observed at any of the exposure levels tested.

Spencer et al. (1951) exposed groups Wistar rats (15/sex/group) for 212 days (up to 151 exposures), randomly bred guinea pigs (8/sex/group) for 162-246 days (121-180 exposures), randomly bred rabbits (2/sex/group)for 232-248 days (165-178 exposures, and rhesus monkeys (2 males/group) for up to 248 days (up to 178 exposures) to 0 (unexposed), 0 (chamber-exposed), 100, 200, or 400 ppm of 99.7% 1,2-dichloroethane 7 hours/day, 5/days/week. Rabbits and monkeys did not receive the mid-level exposures. At 400 ppm, all rats, guinea pigs, and monkeys died or were killed in extremis within 56 (rats), 32 (guinea pigs), or 12 days (monkeys). Adverse effects included weight loss (rats and guinea pigs), fatty livers (rats), fatty liver degeneration (guinea pigs and monkeys), cloudy swelling of the kidney tubular epithelium (guinea pigs), renal tubule degeneration with cast formation (monkeys), and increased liver and kidney weights (guinea pigs). Hematological and clinical chemical blood parameters were unaffected, except for elevated blood nonprotein nitrogen and BUN in the guinea pigs. At 200 ppm, there were no mortalities. Rats were unaffected in growth, organ weights, hematology, clinical chemistry, or histopathology. Guinea pigs showed poorer growth in both sexes, but final body weight was significantly depressed only for males. Half the guinea pigs of both sexes had parenchymatous liver degeneration with fat vacuoles and increased liver lipids. At 100 ppm, there were no mortalities or effects on body or organ weight, hematology, clinical blood chemistry, histopathology, or liver fat.

Hofmann et al. (1971) exposed groups of randomly bred cats (2/sex/group), randomly bred "colored" rabbits (2/sex/group), Pirbright-White guinea pigs (5/sex/group) and Sprague-Dawley rats (5/sex/group) to 0, 100, or 500 ppm 1,2-dichloroethane of >99% purity 6 hours/day, 5 days/week for 6 (high dose) or 17 weeks (low dose). At 500 ppm, the rabbits, guinea pigs, and rats died within 17 exposures. Reported effects included dyspnea (rats), increased serum urea (cats), dilation of the heart (cats and rabbits), hyperemia with some edema of the lungs (rats and guinea pigs), fatty degeneration and necrosis of the myocardium and liver (rats and guinea pigs) and lipoid nephroses and disgorgement of the adrenals (rats and guinea pigs). At 100 ppm, there were no chemically related effects on clinical symptoms, body weight, blood chemistry, or histopathology.

Maltoni et al. (1980) exposed groups of male and female Sprague-Dawley rats and Swiss mice (90/sex/species/group) 5, 10, 50, or 150-250 ppm 99.8% pure 1,2-dichloroethane 7 hours/day, 5 days/week for 78 weeks starting at age 11-12 weeks. Interim sacrifices of 8-10 rats were made after 3, 6, or 18 months exposure. After several days of exposure, the highest

exposure level was decreased to 150 ppm because of severe toxicity (unreported number of deaths). Two control groups of 180 rats (one in an exposure chamber and the other in a nearby room) and one group of 249 mice were examined. Additional groups of 8-10 rats were started at age 14 months and exposed 12 months to 0, 5, 10, 50, or 150 ppm for blood work-ups (Spreafico et al., 1980) and histopathology examinations (data not available). With the exception of the increased mortality in rats exposed to 250 ppm, no concentration related effect on mortality was observed in the rats. Female high-dose mice appeared to have slightly higher mortality during the first 80 weeks. This study was designed to assess the carcinogenicity of 1,2-dichloroethane and non-carcinogenic histopathological alterations were not reported. No concentration related changes in SGOT, SGPT, gamma-glutamyl transpeptidase, bilirubin, cholesterol, alkaline phosphatase, lactic acid dehydrogenase (LDH), creatine phosphokinase, BUN, and uric acid were observed in rats exposed to 1,2-dichloroethane for 3, 6 or 18 months (Spreafico et al., 1980). However, in rats that were exposed for 12 months beginning at age 14 months, the following alterations in clinical chemistry values were observed: increased SGOT levels in the 5 and 10 ppm groups, but a decrease at 50 and 150 ppm; increased SGPT and gamma glutamil transpeptidase levels at 50 and 150 ppm; decreased serum cholesterol levels at 50 and 150 ppm; decreased LDH levels at 5 ppm or greater; and increased uric acid levels at 50 and 150 ppm. The clinical chemistry data from the rats exposed for 12 months starting at age 14 months, is suggestive of liver and possibly kidney toxicity.

The immunotoxic effects of inhaled 1,2-dichloroethane in young male Sprague-Dawley rats and young female CD-1 mice were examined by Sherwood et al. (1987). The rats were exposed for 3 or 5 hours to 0, 100, or 200 ppm (0, 405, or 809 mg/m^3) 1,2-dichloroethane or to 0, 10, 20, 50, or 100 ppm (0, 40.5, 81, 203, or 405 mg/m^3) 1,2-dichloroethane 5 hours/day, 5 days/week for 12 exposures. The mice were exposed to 0 or 2.5 ppm (10.1 mg/m^3) 1,2-dichloroethane (purity not reported) 3 hours/day for 5 days. Additional mice were exposed to 0, 2.5, 5, 10, or 100 ppm (0, 10.1, 20.2, 40.5, or 405 mg/m^3) 1,2-dichloroethane for a single 3-hour period. Testing was conducted after the single or repeated exposure periods terminated. The number of animals/species/exposure level varied with the test, ranging from 5 for alveolar macrophage cytostasis in rats to 140 for mortality from streptococcal pneumonia in mice.

In rats, no effects were observed on pulmonary bactericidal activity to inhaled Klebsiella pneumonia, in vitro phagocytotic activity of alveolar macrophages, cytostatic and cytolytic capacity of alveolar macrophages, alveolar macrophage ectoenzymes activity, or mitogenic stimulation of lymphocytes from lung-associated, mesenteric or popliteal lymph nodes. In mice, a single 3-hour exposure to 5 or 10 ppm 1,2-dichloroethane significantly ($p<0.05$ at 5 ppm, $p<0.001$ at 10 ppm) increased mortality (monitored over a 14-day post exposure period), relative to controls, due to a exposure to an aerosol of viable Streptococcus zooepidemicus. However, a single exposure or repeated exposures to 2.5 ppm had no significant effects. An exposure level of 10 ppm 1,2-dichloroethane significantly decreased bactericidal activity towards inhaled K. pneumonia; lower exposure levels were without effect. Single exposure to 10 or 100 ppm 1,2-dichloroethane did not affect the total numbers or differential counts of cells recovered by pulmonary lavage, or the phagocytic or cytostatic

ability of the alveolar macrophages in vitro. In this study, the 2.5 ppm (10.1 mg/m^3) exposure level represents a NOAEL for immunotoxicity in mice, whereas 5 ppm (20.2 mg/m^3) is a LOAEL. In rats, the highest exposure level, 200 ppm (809 mg/m^3), 5 hour/day, 5 days/week for 12 exposure periods, may represent a NOAEL.

Of importance in the derivation of criteria for 1,2-dichloroethane is the interpretation of the immunological results reported by Sherwood et al. (1987). In that study, the mortality incidence after a challenge with S. zooepidemicus was significantly increased in mice exposed to 5 ppm (20.2 mg/m^3) 1,2-dichloroethane for a single 3-hour period. Exposure to 2.5 ppm (10.1 mg/m^3) for a single or five daily periods was without effect. Although the 3-hour exposure duration appears inappropriate for derivation of either a 1-2-day AIC or a 30-day AIC, the study by Sherwood et al. (1987) must be taken into consideration. At issue, however, is the relevance of the effect to humans. In support of the results of Sherwood et al. (1987), it must be mentioned that evidence of suppressed humoral and cell-mediated immunity was seen in mice treated orally by gavage for 14 days with 1/100 the LD50 for 1,2-dichloroethane (Munson et al., 1982); however, 90 days of treatment with 4/10 the LD50 in the drinking water had no significant effects on the same endpoints. Munson et al. (1982) attributed the difference between their short- and long-term results partly to the different administration protocol and/or to the fact that 1,2-dichloroethane may have induced its metabolism over the longer exposure period. It must be noted that neither Sherwood et al. (1987) nor Munson et al. (1982) monitored serum steroids, which are known to influence immunocompetence. The relevance of the challenge with S. zooepidemicus can be further questioned on the grounds that in several longer-term inhalation exposure studies (summarized in the previous section), in which exposure levels were much greater than those used by Sherwood et al. (1987), no apparent increased death rates due to infection were observed in 1,2-dichloroethane-treated animals (several species including mice).
Although the Sherwood et al. (1987) study is a short-term study, it reports effects at very low exposure levels, indicating a need for further investigation of the potential immunotoxicity of 1,2-dichloroethane.

D. Studies of developmental or reproductive toxicity: U.S. EPA (1985a) reviewed the database on the reproductive and developmental toxicity of 1,2-dichloroethane and concluded that 1,2-dichloroethane does not have the potential to produce adverse reproductive or developmental effects at maternally non-toxic levels.

Schlachter et al. (1979) exposed groups of pregnant Sprague-Dawley rats (16-30/group) and New Zealand rabbits (19-21/group) to 0, 100, or 300 ppm of 99.9% 1,2-dichloroethane 7 hours/day on gestation days 6-15 (rats) or 6-18 (rabbits). Increased incidences of maternal mortality were observed in rats and rabbits exposed to either concentration of 1,2-dichloroethane. Decreased maternal weight gain was observed in rat dams exposed to 300 ppm and increased relative liver weight in rat dams exposed to 300 ppm and rabbit does exposed to 100 or 300 ppm. In both species, maternal toxicity was associated with resorptions and/or early delivery. In the offspring of survivors, malformations or other signs of toxicity were not observed.

In a 1-generation study of reproductive toxicity, Sprague-Dawley rats (20-30/sex/group) inhaled 0, 25, 75, 150 ppm of 99.98% 1,2-dichloroethane 6 hours/day, 5 days/week for 12 weeks, after which they were mated to produced two liters (Rao et al., 1980). Exposures of the females continued through breeding, gestation, and lactation. There was an exposure-related increase in absolute liver weight in the males, but absolute liver weight was only significantly increased in the females of the parental generation exposed to the highest concentration. Other adverse effects appeared more related to concurrent disease than to 1,2-dichloroethane exposure. The fertility and survival indices, sex ratios, the number of live pups per litter, neonatal body weight, and malformations were not related to 1,2-dichloroethane exposure.

In a two generation reproduction study, groups of 10 male and 30 female ICR Swiss mice were provided with drinking water containing 0, 0.03, 0.09, or 0.29 mg/ml 1,2-dichloroethane for 35 days before mating to produce 3 litters (Lane et al., 1982). Offspring were selected to breed 1-2 litters. Exposures continued throughout the experiments. Developmental studies were conducted on females of the F1 and F2 generations. There were no adverse compound-related effects on body weights, gross pathology of internal organs, letter sizes, or pup body weight, survival, or malformations. U.S. EPA (1985a) regarded the study as inconclusive, since the doses were insufficient to produce parental toxicity at any level.

U.S. EPA (1985a) reviewed a number of Soviet studies which reported reproductive effects in humans following exposure to 1,2-dichloroethane. Because of poor reporting of results, U.S. EPA (1985a) felt that the studies could not critically evaluated. Urusova (1953) reported accumulation of 1,2-dichloroethane in unreported numbers of nursing women occupationally exposed to unreported concentrations of 1,2-dichloroethane. No data were located reporting adverse effects on offspring consuming 1,2-dichloroethane contaminated milk.

2. Derivation of a provisional RfC:

The human and animal data for 1,2-dichloroethane suggest that the gastrointestinal tract, liver, possibly the kidney, and mucous membranes are targets of 1,2-dichloroethane toxicity. Two studies were considered for the derivation of a provisional RfC for 1,2-dichloroethane (Kozik, 1957; Spreafico et al, 1980). An increased incidence of gastrointestinal disturbances and liver and gallbladder disease were observed in workers exposed to 10-15 ppm (40-61 mg/m^3, assuming 25 C and 760 mm Hg) (Kozik, 1957). As discussed previously, there are a number of limitations to this study, including the lack of statistical analysis of the data, information on the duration of exposure, and control of potentially confounding factors. In animals, exposure to 50 ppm (202 mg/m^3) results in a statistically significant increase in the levels of several serum enzymes, indicative of liver and/or kidney damage (Spreafico et al., 1980). The NOAEL identified in this study is 10 ppm (40 mg/m^3). In this study, histopathology results of non-carcinogenic lesions were not reported and the respiratory tract was not completely examined (Maltoni et al., 1980). Of these two studies, the Kozik (1957) is considered the more appropriate basis for a provisional

RfC. The LOAEL identified in this study is 10 ppm (40 mg/m^3). An equivalent continuous exposure level (LOAEL(HEC)) of 14 mg/m^3 is estimated by assuming occupationally exposed humans inhale 10 m^3/8-hour workday and 10 m^3 during the remainder of the day, and work 5 day/week.

$$LOAEL(HEC) = 40\ mg/m^3 \times (10\ m^3 / 20\ m^3) \times (5\ day/7\ day)$$

Application of an uncertainty factor of 3000 (10 for use of a LOAEL, 10 to for use of a less than chronic study, 10 to protect sensitive individuals, and 3 due to deficiencies in the database such as the incomplete evaluation of potential respiratory effects and uncertainty regarding the relevance to humans of the immunological data in mice) yields a provisional RfC of 5E-3 mg/m^3. Because Kozik (1957) did not report the duration of exposure, the most conservative approach would be to assume that the exposure was less than chronic. Thus, an uncertainty factor 10 was applied to account for use of a less than chronic study. Because of deficiencies in the principal study and database, low confidence is placed in this provisional RfC of 5E-3 mg/m^3.

0.0014 mg/kg/day

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Attachment 5

Risk Assessment Issue Paper for:
Carcinogenicity Information for
Tetrachloroethylene (perchloroethylene, PERC) (CASRN 127-18-4)

The carcinogenicity characterization has a long history. A July 1985 Health Assessment Document for Tetrachloroethylene (Perchloroethylene), EPA # 600/8-82/005F, classified the agent in Weight-of-Evidence Group "C - Possible Human Carcinogen" mentioning that this would be reevaluated because of new information. The 1985 document also provided upper bound inhalation and oral risk estimates. An April 1987 Addendum to the Health Assessment Document, EPA# 600/8-82/005FA, proposed that the Weight-of-Evidence be upgraded to "B2 - Probable Human Carcinogen" and provided a revised inhalation risk estimate. A February 1991 document titled Response to Issues and Data Submissions on the Carcinogenicity of Tetrachloroethylene, EPA# 600/6-91/002A discussed newer data relative to weight-of-evidence classification. The Agency's Science Advisory Board has reviewed these documents finding them to be technically adequate while offering an opinion that the weight-of-evidence is on C-B2 continuum (C=Possible Human Carcinogen, B2=Probable Human Carcinogen). At present time, the Agency has not adopted a final position on the weight-of-evidence classification.

The upper bound risk estimates from the 1985 Health Assessment Document as amended by updated inhalation values from the 1987 Addendum have not as yet been verified by the IRIS-CRAVE Workgroup. The estimates are viewed as useful information in the context of the information available in the 1985-1987 period.

ORAL: 1985 HAD; Unit risk = 1.5E-6 per ug/L

Slope Factor = 5.2E-2 per mg/kg/day

INHALATION: 1987 Addendum; Unit risk = range form 2.9E-7 to 9.5E-7 with a geometric mean of 5.8E-7 per ug/cu.m

Slope factor = 2.0E-3 per mg/kg/day

Those needing to make a choice about carcinogenicity have found the 1985, 1987 and 1991 EPA documents and the 1988 and 1991 Science Advisory Board letters of advice useful background information. When the Agency makes a decision about weight-of-evidence, the CRAVE-IRIS verification will be completed and the information put on IRIS.

Attachment 6

(07-02-92/OHEA)

Risk Assessment Issue Paper for:
Carcinogenicity Information for Styrene
(CASRN 100-42-5)

Recent efforts to characterize the presence or absence of a carcinogen potential for styrene monomer go back to a January 1988 Drinking Water Criteria Document for Styrene, EPA# ECAO-C-409 and an October 1989 Health Effects Assessment Document, EPA# 600/8-88/054. The Agency's Science Advisory Board offered advice on the carcinogenicity weight-of-evidence classification in 1988 and 1990.

At the present time, the Agency has not decided how to describe the carcinogenicity evidence. Those needing a position may find the International Agency for Research on Cancer (IARC) view useful. IARC has classified styrene as a "Possible" human Carcinogen according to their classification criteria because of positive but limited animal data. Traditionally, IARC does not attempt to provide estimates of cancer unit risk or potency.

When the Agency adopts a carcinogenicity characterization for styrene, the information will be entered into IRIS.